The future is digital

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The future is digital: *In silico* tissue engineering

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Abstract

The Industry 4.0 concept refers to automation and data exchange in manufacturing technologies, which includes technologies for cell therapy product manufacturing. An important aspect of this concept is the development and use of Digital Twins. A Digital Twin is a digital representation of a product or process that is used to optimize the design and use of said product or process. In this opinion article, we show that such Digital Twins have already been developed for a variety of tissue engineering processes. Using skeletal tissue engineering as a case study, we discuss a number of models at various stages of use between bench and bedside and ranging from pure data-driven models to models built on known mechanisms and first principles. Finally, we emphasize the importance of data collection and model validation to ensure, amongst others, compliance to regulatory guidelines.

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Abbreviations

ATMP, Advanced Therapeutic Medicinal Product; ASME, American Society of Mechanical Engineers; CAD-CAM, Computer Aided Design – Computer Aided Manufacturing; cBiT, compendium for Biomaterial Transcriptomics; CFR, Code of Federal Regulations; CQA, Critical Quality Attribute; DoE, Design of Experiments; EU, European Union; FDA, Food and Drug Administration; GDPR, General Data Protection Regulation; GMP, Good Manufacturing Practices; QbD, Quality by Design; TE, Tissue Engineering; VVUQ, Verification Validation & Uncertainty Quantification.

Introduction

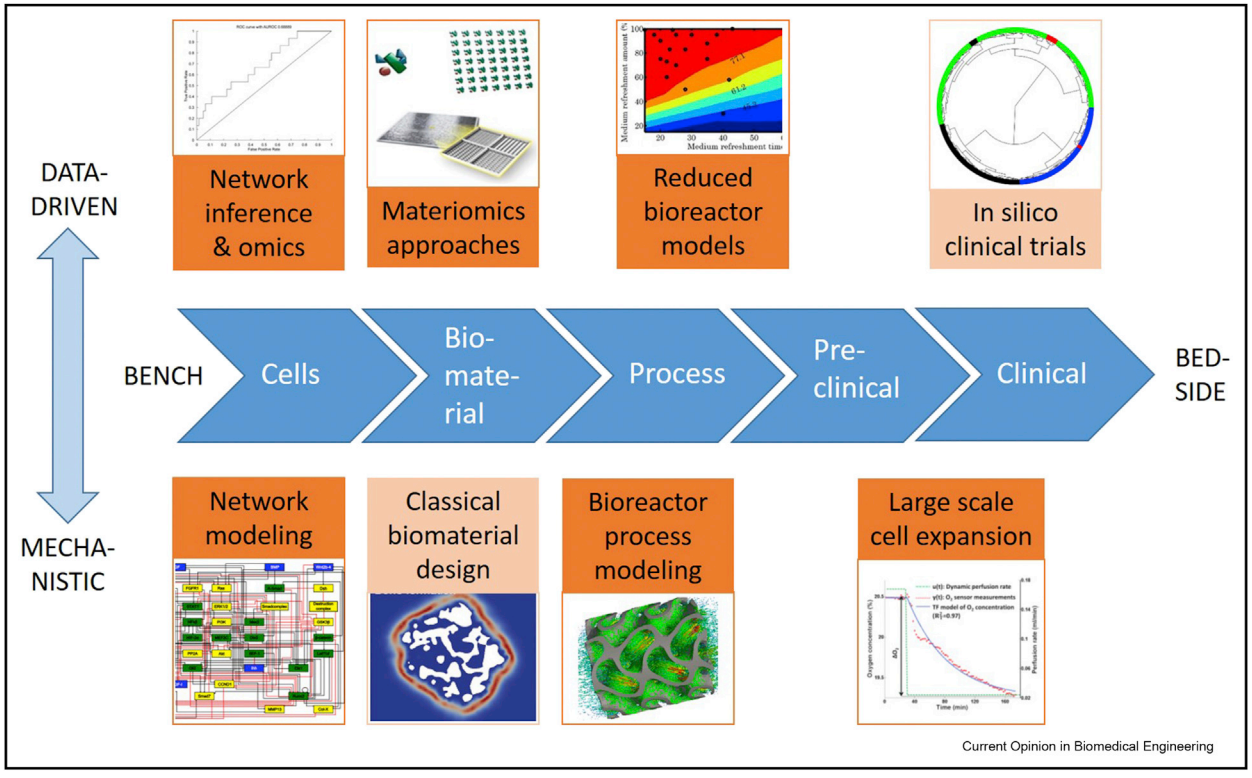
Having entered the 4th phase of the industrial revolution, so-called Industry 4.0 or smart industry, automation and data exchange have become crucial concepts in modern manufacturing technologies [1]. Industry 4.0 creates a 'smart factory' and within such factories, cyber-physical systems monitor physical processes, creating a virtual copy of the physical world that subsequently can be used to make decentralized decisions. These cyber-physical systems communicate and cooperate with one another and with humans via the 'Internet of Things' and cloud computing. In the context of Industry 4.0, another engineering concept that has been around for 30 odd years in the engineering community, that of the 'Digital Twin', is receiving renewed attention. Whereas originally, the Digital Twin referred to a static representation of physical objects by means of 3D computer models (Computer Aided Design & Manufacturing CAD-CAM), it has extended now to representations of 4D dynamic systems ranging from tiny sensors to huge Boeing 777s and from cars to power plants. The Digital Twin concept provides the engineering community with a way to handle increasing complexity in the design, manufacturing, control and maintenance of objects, systems and processes, which makes it a crucial element for the implementation of the Industry 4.0 concept. This renewed attention for the concept has even lead to it being listed it on Gartner's Top 10 list of Strategic Technology Trends for 2017 as well as 2018 [2].

Industry 4.0 is by no means restricted to classical engineering industries. On the contrary, many biotech companies are already fully committed. Nevertheless, in regenerative medicine there is still substantial progress to be made. Cell-based therapies have often been presented as potential major growth drivers of the global economy. With a record number of ongoing clinical trials, the field has emerged from the 'valley of death' it went through in the late '90s, a period that is typical for new emerging technologies that are hyped beyond what they are able to deliver at that stage. One of the major bottlenecks and cost drivers at this point is the manufacturing process that all too often is a scaled out version of the cumbersome manual laboratory procedures established during the development phase. Embracing the concepts of process engineering that since long are being used in biopharmaceutical

engineering, and linking up to the Industry 4.0 trend would allow the field to increase product quality and productivity, decrease cost and increase the (economic) viability of the products.

As mentioned above, one of the important steps in order to fully embrace the Industry 4.0 concept is the development of Digital Twins. The complexity required from a Digital Twin will vary based on the use case and the business objective. Whereas in certain situations Digital Twins can be as simple as read-outs of specific functional or technical parameters, in other cases they might require a highly detailed mechanistic digital representation of the process. Digital tools can be classified in a number of ways. One of them is according to the *in silico* (modelling) technology they are based on (see Figure 1, vertical), ranging from data driven technologies (top) where only experimentally generated information is used, to mechanistic modelling technologies (bottom) that have been developed based on concepts and hypotheses informed by current biological knowledge and insights. Mechanistic models may be superior in providing insight into the system under consideration, since their parameters have a physical meaning. However, they have a significant cost of development, are more difficult to parameterise, and are generally harder to compute in real-time, which is often a requirement for Digital Twins. Furthermore, the complexity of the biological processes is often too high, or our understanding of the process is too limited, in order to describe them by fully mechanistic models. Because of their black-box nature, data-driven models on the other hand provide less insight into the system, but are more straightforward to develop, and in situations where the data logging surpasses the speed of analysis they, provide an ideal basis for online prediction and control [3]. In the field of regenerative medicine, there is often abundant high-quality manufacturing process data available (O_2, pH etc.) due to GMP requirements, which could be an advantage for the data-driven approaches. Nevertheless, doctors and regulators might be reluctant to use data-driven models in clinical settings due to their black-box nature. Additionally, results from a data-based model can often not be extrapolated to cases outside of the initial scope of the data. Ultimately, hybrid strategies, resulting in the formation of a cross talk and integration between data-based and mechanistic models could provide an efficient framework to gain the best of both worlds [4].

Figure 1



Schematic representation of the tissue engineering R&D process (horizontally) and the computer model classification (vertically). The dark orange boxes illustrate the examples discussed in this paper: cellular regulation, materiomics, bioreactor process quantification & cell expansion. Examples in the light orange boxes (classical biomaterial design [5] & *in silico* clinical trials [6] are not discussed in this paper, but are mentioned to complete the overview. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

In this opinion paper, we will focus on the presentation of a number of digital strategies developed in the field of regenerative medicine linking straight into the concept of the Digital Twin. We will discuss strategies, both data-driven and mechanistic, some of which are mostly confined to the research phase of the tissue engineering pipeline whereas others that are mostly relevant in the development phase (Fig. 1, horizontal). For the sake of consistency and given the authors' expertise, the specific examples discussed originate from the sub-field of skeletal tissue engineering. However, some of the tools are generic in terms of the biological applications, whereas for other tools tissue-specific counterparts in other organ systems also exist or are being developed. The word "process" is used throughout the text in a variety of meanings, referring to the biological process (of cell proliferation, cell differentiation etc.), the bioreactor process, the manufacturing process (all steps leading up to the production of the TE product) or the process of computer modelling (involving design, implementation and validation).

Examples

Starting at the bench side of the R&D pipeline, the first example describes the use of a literature-derived network model to gain more insight in the myriad interactions governing the behaviour of a growth plate chondrocyte. The following example discusses the use of high-throughput material surface topography testing devices with the appropriate digital tools to quantify and interpret the results. A third example shows how mechanistic models can help to quantify the microenvironment cells create for themselves during culture in a perfusion bioreactor set-up while the last example discusses data-based digital tools that can be used to monitor and control the bioreactor process itself.

Cellular regulation of the growth plate as a blueprint for skeletal stem cell culture strategies

The specialization of cartilage cells, or chondrogenic differentiation, is an intricate and meticulously regulated biological process that plays a vital role in both bone formation and cartilage regeneration. Understanding the molecular regulation of this process allows optimizing cell culture and differentiation strategies that are used in the context of skeletal tissue engineering. The studies by Kerkhofs et al. [7–9] describe the development of a network model of the growth plate chondrocyte based on studies reported in the literature. This literature-based network contains the major pathways active in the growth plate. In a first implementation, a Boolean modelling strategy was used where genes can be either on or off, interactions amongst the genes are described by Boolean operators and the notion of time is completely absent [7]. Despite these simplifications, the model was able to capture the general behaviour of the chondrocytes in the different layers of the growth plate. Furthermore, the effects of various knockouts on the growth place signalling and morphology were accurately captured by the model.

In a follow-up study [8], the Boolean modelling strategy was abandoned for an additive strategy, allowing genes to take any value between 0 and 1 as well as having some time resolution by making a distinction between fast (post translational modification) and slow (transcriptional) processes. These new features allowed capturing the dose effect of certain growth factor actions. Subsequently, the model was used to perform an *in silico* screening of the contribution of all of the modelled factors towards the different chondrocyte phenotypes. In other words, it allowed performing an *in silico* screening of potential culture strategies for either inducing hypertrophy or maintaining cells in a proliferative state [9]. Performing such an *in silico* screening step allows to identify key candidates, which strongly reduced the size and complexity of the experimental screening effort.

To fine-tune the model, a genetic algorithm strategy was used to find those combinations of model parameters (presence and weight of the arrows connecting different players in the network) that allowed capturing overall growth plate expression profiles. Additionally, a variety of network inference methods was used in a consensus approach to infer a regulatory network from microarray studies published in the literature. This consensus network served to identify possible additions to the network for which insufficient direct evidence was available in the literature at the time of the construction of the literature-derived network, and which might have interesting therapeutic consequences.

A systems approach to the design of new biomaterials

A systems approach as the one described in the previous example is not only applicable to a pure biological context, but also to the context of materials design, in a field that has been baptized 'materiomics' [10]. Materials and their environments are approached as systems that can be modelled and thus explored *in silico*. That means going beyond the pure material screening towards a more rational material discovery approach. Given the size of the materiome to be explored, moving from low-throughput to high-throughput should indeed entail more than simply testing more materials at the same time. The TopoChip [11] is an example of such a high throughput screening platform where *in silico* tools are used both in the design and the analysis of the screening experiment.

In brief, surface topography has been observed to significantly affect cell shape (roundness, nucleus size, perimeter and orientation) [12], exerts mitogenic

effects [13] and modulates pluripotency [14], however the underlying mechanisms are only partially understood. Using three types of primitive shapes, being triangles, circles and rectangles, a library of topographies was designed by varying the size, number and combination of primitives to be used. These topographies were subsequently imprinted on polylactic acid chips and seeded with cells in a static culture experiment. The cell–material interactions on the chip could be quantified using a plethora of techniques from high-throughput gene expression to imaging assays. For the latter, automated high content imaging on the basis specific fluorescent markers was the starting point of the analysis, followed by the use of CellProfiler's automatic imaging pipeline for data quality check and segmentation. The resulting huge set of quantitative data was mined using machine learning and computer modelling. So far, analyses have revealed a spectrum of topographies that induce various levels of cellular morphologies [11] and topographies that stimulate proliferation and induced pluripotent stem cell pluripotency [15].

3D neotissue growth modelling for understanding and optimising bioreactor culture processes

Topography is not only important for the cells' response to the material surface, but also to the geometry of the 3D scaffold they are seeded on. 3D scaffold-based cell growth for the formation of engineered tissues in perfusion bioreactors is a highly complex process with multiple parameters affecting the properties of the end product. In order to be able to implement design principles, *in silico* tools are required that able to capture and subsequently steer such highly complex processes. Briefly, scaffold topography parameters such as local curvature can affect tissue growth [16] a phenomenon attributed to the tensile forces that can be sensed by cells in such locations [17]. In addition, tissue growth kinetics can be influenced by flow related stresses as well as concentrations of nutrients, growth factors, and metabolic byproducts. Guyot et al. [18] used the level-set method for simulating this curvature-dependent growth of neotissue (cells and their extracellular matrix) by tracking the evolution of the interface between the neotissue and the void space on the three-dimensional scaffold, and by using image based strategies based on nanofocus Computed Tomography technologies [19]. Through this initial *in silico* tool previous experimental findings [20] were confirmed, showing the relevance of the model. In subsequent studies, this model was extended to include the impact of parameters associated with dynamic culture conditions by incorporating the Brinkman equations governing the flow. This allowed quantifying fluid velocity and shear stress profiles for both free flow and flow at the tissue interface, but also within the tissue's porous domain [21]. This was a progress with respect to the state of the art where shear stress calculations were carried out on empty scaffolds [22,23] or assuming neotissue to be an impermeable volume without any flow [24,25]. It also allowed whole scaffold volumes to be captured and not only small a compartment of the domain. The aforementioned approaches including cells on the scaffold domain were sufficient for capturing the initial stages of neotissue formation. However, they were inadequate for capturing the later stages as the growing neotissue is a porous tissue that allows flow through its own micro-pores, thereby changing the local flow environment as well as the mechanical (shear) stimulation on the cells inside the neotissue, and thereby ultimately affecting the neotissue growth itself. Therefore, in a next modelling step, growth kinetics of the *in silico* neotissue were coupled to the mechanical factors [26] but also to the physicochemical factors (unpublished Guyot) thus constituting a model that could substantially aid in deciphering and subsequently designing bioreactor processes.

The application of mechanistic models such as the one described above can be hampered by their high-cost. Although "accurate" information can be extracted, it would not be possible to use them for investigating a large process space. Hence model reduction strategies have been also explored. Mehrian et al. [27], applied model reduction by reformulating the neotissue growth model discussed above from a set of partial differential equations into a set of ordinary differential equations. By following this approach, a homogenized model was obtained that was 10^5 fold faster than the mechanistic 3D version, which permitted the application of rigorous optimization techniques such as Bayesian optimization. This allowed a "high throughput" *in silico* screening that determined global optima for the medium refreshment strategies in terms of frequency and percentage of medium replaced, leading to maximized neotissue growth kinetics in the perfusion bioreactor set-up under study.

On-line monitoring techniques for critical quality attributes of cells

For cell therapy bioreactors, in general, it is common that on-line measurements of physicochemical variables (e.g. pH, O_2) are used to control the environmental conditions. However it is more challenging to obtain on-line and non-invasive information directly on the critical quality attributes (CQAs) of the cells themselves. Even more challenging is to use this information to directly control the CQAs of the cultured cells, e.g. their growth characteristics, metabolic state, and ultimately *in vivo* potency. As more process data is collected and the effect of the microenvironment on the cells is better understood, it is a promising approach to estimate the unmeasurable CQAs in real-time, based on (a combination of) indirect measurements that can be related to the CQA of interest. This strategy, where the information of

(multiple) indirect data streams is translated into interpretable process parameters is generally known as a 'soft sensor' method [28,29]. Ultimately this 'model-based monitoring' strategy, if combined with a desired output trajectory of the CQA, can be used as the basis for 'model-based control' in which the measured process data and a model are used to determine the most appropriate controller setting to reach the desired state of the process or the cells. More often used in fermenter bioreactors [30–33], only limited examples of model-based control can be found in literature for stem cell bioreactors [34]. An example of model-based predictive monitoring can be found in Ref. [35] where a data-driven model based on real-time imaging data from a bioreactor's harvest process was able to link the on-line monitored feature variable (cell circularity) to a process decision ('stop the enzymatic harvest reaction'). The online monitoring and data-analysis allowed even to predict when to stop the cell harvesting process, while the process was still ongoing (i.e. making the monitoring faster than real-time). The data-driven monitoring method used in this application is useful to optimise the incubation time for cell harvests in planar culture systems for different donors or different harvest solutions. Another example of this data-based modelling, this time in a perfusion bioreactor, can be found in Ref. [36], where a purely data-driven transfer function model was used to relate dynamics in oxygen measurements to estimations on the number of cells present in the bioreactor. However, this data-driven model might not be applicable outside of the scope for which it was originally developed. Therefore, in order to corroborate the results of the data-driven model, the steady state gain of the transfer function was linked to the dynamics in the oxygen data by means of a simple mechanistic oxygen consumption model. Another hybrid modelling strategy could be to start from the mechanistic model and perform a model reduction step, followed by a re-parametrisation based on available experimental data, as has been described in the previous section [27]. Similar approaches based on this online data-based monitoring and control strategy pose tremendous potential if one would have access to online readouts of biomarkers that make up the biological signature of a cellular state and therefore enable the control of *in vivo* cell potency *in vitro*.

Discussion

When developing and using digital technologies for biomedical applications, there are a number of considerations to be made regarding various steps of the modelling process. Data collection and validation are two extremely important steps that are currently the subject of intense efforts within the community. Furthermore, interoperability, cloud computing & infrastructure and the implications of the General Data Protection Regulation (GDPR) and open science initiatives are important to consider but their discussion is beyond the scope of this paper.

Data collection

A major prerequisite for *in silico* models is the access to large amounts of qualitative data, either to construct the data-driven models or to validate the mechanistic models. Data platform initiatives could help to collect structured data for the development of *in silico* models. In R&D settings (towards the bench side of Fig. 1) often experimental data is still collected with respect to one specific experimental variable. This one-factor-at-a-time approach yields data for models with a very limited scope. More recently, by virtue of the quality-by-design (QbD) principles that are becoming mainstream in regenerative medicine development [37], data collection taking the whole design space into account by a Design of Experiment (DoE) approach yield much richer datasets. Subsequently, the resulting models are often able to describe the biological processes more realistically. Since well characterized experimental results could serve multiple modelling purposes, data platform initiatives such as the compendium for Biomaterial Transcriptomics (cBiT) for materials design [38] could reduce the effort of model development and validation significantly.

Closer to the bedside, in the regulated environment of regenerative medicine applications, large amounts of data are being collected, for example to comply to Good Manufacturing Practices (GMP). While currently this data is generally primarily collected for regulatory purposes, they contain a wealth of information for *in silico* models. However, often the data structure and medium (i.e. paper formats) in a GMP setting are not always ideal for modelling purposes. Additionally the labour cost of acquiring, or even logging, additional data in a GMP setting is very high. Processes with on-line in-process controls are therefore not only beneficial for the product quality, but also as a basis to develop *in silico* models. With the upcoming Internet-of-Things technology, the efficient and regulatory compliant (e.g. 21 CFR part 11 [39] and EU GMP Annex 11 [40]) collection and subsequent analysis of this data becomes more important. Again, data platform initiatives do exist that try to consolidate the (pre-) clinical data streams in regenerative medicine (e.g. MyCellHub, Trakcel, Vineti,…).

Model validation

In silico models can be (and already are)- part of dossiers that are submitted to the regulator, either in the form of evidence obtained from *in silico* modelling or in the form of software that is an integral part of the device that needs to be regulated. This requires the sponsors to demonstrate the credibility of their *in silico* tools. The first step in building this credibility is documentation of

the model and its implementation. The USA Food and Drug Administration (FDA) has published a clear guidance [41] explaining what technical information needs to be communicated when submitting (the results of) a digital tool. The next step is the VVUQ - verification, validation and uncertainty quantification. Whereas verification refers to the correspondence between the simulation outcome and the underlying mathematical equations, validation refers to the correspondence between the simulation results and the real-world observations. In a joint effort between the FDA and the ASME (American Society of Mechanical Engineers), guidelines are being formulated in the form of a verification & validation standard for medical devices (V&V40), in imitation of the V&V's that have been developed for *in silico* products from the automotive and aerospace industry. Uncertainty Quantification looks at how the uncertainties on the model inputs and assumptions effect the simulation results. A last step in the credibility building process is the applicability analysis, looking at the relevance of the computer model and its validation evidence to the proposed context of use [42]. Even though most of the concepts mentioned in this overview have been applied primarily to medical devices and, to a lesser extent, to drugs, the same strategy applies to advanced therapy products (ATMP).

Summary and outlook

One of the key elements of the Industry 4.0 initiative lies the vision of a digital transformation of manufacturing (i.e. the Digital Twin), especially when high value products come in play such as in the case of tissue engineering. For example, the implementation of "smart" self-regulated factories, able to guide cellular transformations and ATMP assembly using feedback signals can only become a reality by the adoption of *in silico* elements for monitoring and control. Subsequently, the ability to systematically and rigorously optimize tissue engineering processes using *in silico* tools can allow process intensification and drive production footprint to a minimum, an element which is highly necessary for costly GMP-grade AMTP manufacturing. From initial cell culture, biomaterial development and process design, model-based approaches should be adopted to minimize risk and cost of ATMP development. With the adoption of novel automated downstream units of operation adapted for use in the cell therapy and regenerative medicine field, digitised process integration and whole process self-regulation can become a reality. Tissue engineering could thereby become a major industrial example where the industry 4.0 vision and the incorporation of the Digital Twin concept becomes not just an improvement, but a necessity.

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Conflict of interest

TL is affiliated with MyCellHub. LG, AC & IP declare no conflict of interest.

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Presentation of a novel framework for performing applicability analysis () and demonstrate its use with a medical device computational model. The framework provides a systematic, step-by-step method for breaking down the broad question of applicability into a series of focused questions, which may be addressed using supporting evidence and subject matter expertise. The framework can be used for model justification, model assessment, and validation planning.